INFECTION AND IMMUNITY, Nov. 1985, p. 577–582
0019-9567/85/110577-06$02.00/0

Vol. 50, No. 2

Flagella and Motility Alterations in *Pseudomonas aeruginosa* Strains from Patients with Cystic Fibrosis: Relationship to Patient Clinical Condition

MARY ANNE LUZAR,[1] MARY JANE THOMASSEN,[2]* AND THOMAS C. MONTIE[1]

Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37996-0845,[1] and Departments of Pediatrics and Microbiology, Case Western Reserve University School of Medicine and Rainbow Babies and Childrens Hospital, Cleveland, Ohio 44106[2]

Received 19 April 1985/Accepted 7 August 1985

Selected physiological parameters of 31 classic and rough *Pseudomonas aeruginosa* strains from respiratory tract cultures of patients with cystic fibrosis were examined. An association of a patient's clinical condition (good or poor) with strain physiology was made. Rough strains from patients in poor clinical condition demonstrated severe alterations in motility when compared with M-2, a highly motile and chemotactic burn strain. Of the 10 rough strains from patients in poor clinical condition, 70% lacked flagella, as determined by electron microscopy. The remaining few flagellated strains from this group exhibited weak motility both in soft agar and by the capillary assay. Their chemotactic response to three amino acids, when compared with that of strain M-2, was reduced approximately 30 to 90%. Classic strains from patients in poor clinical condition were less chemotactic than those from patients in good clinical condition. A majority of classic and rough strains from patients in good clinical condition were comparable to M-2 in both chemotaxis and motility. Changes in other physiological characteristics indicated by reduced growth rates, or auxotrophy, were seldom observed in the cystic fibrosis strains studied. The data suggest that host-selective pressures, associated primarily with patients with cystic fibrosis that are in poor clinical condition, result in the loss of factors related to invasiveness such as motility and chemotaxis. We propose that these results may reflect that there is a more general alteration in the cell envelope of cystic fibrosis strains.

Pseudomonas aeruginosa has emerged as the major pathogen associated with cystic fibrosis (CF) lung pathology (7, 12, 13, 21, 23, 32, 34, 36). Three morphological types of the organism predominate in the lungs of patients with CF: classic (irregular shape with a smooth, matte surface), rough (irregular shape with rough, matte surface), and mucoid (circular shape with smooth, shining surface) (38). Although much significance has been placed on the importance and frequency of *P. aeruginosa* mucoid strains in CF (9, 12, 19), as an initial study we specifically studied nonmucoid strains. Results of research by many investigators (4, 8, 10, 27) have indicated that mucoidy of *P. aeruginosa* is an inherently unstable characteristic which may be strongly influenced by the laboratory environment.

Many *Pseudomonas* exoproducts such as proteases, exotoxins, and phospholipases, known to play an important role in certain acute infections (33), currently are being considered as factors which may promote CF lung cytopathology (15, 16). In addition to these exoproducts, certain physiological characteristics, such as motility and chemotaxis, may shed light on the subject of persistence. Results of studies with isogenic mutants for chemotaxis and motility indicate that these strains are avirulent in the burned mouse model (D. Drake, G. Shaw, and T. C. Montie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, B 155, p. 43). Some evidence now exists which suggests that certain physiological characteristics of *P. aeruginosa*, associated with its virulence in acute infections may, in fact, undergo alterations in the state of chronic infection in the lungs of patients with CF (11, 25, 35).

We examined the physiological parameters of motility and chemotaxis in classic and rough strains from patients in good (G) and poor (P) clinical condition in an attempt to answer the following questions. First, after initial lung invasion by the pathogen, is there progressive host selection against bacterial properties normally associated with invasive virulence? Second, what is the phenotype of *P. aeruginosa* associated with chronic CF infection in the lungs?

MATERIALS AND METHODS

Bacteria and culture media. All *P. aeruginosa* strains described in Table 1 were isolated from respiratory tract cultures of patients with CF at the Cleveland CF center. *P. aeruginosa* M-2 was originally isolated from the small intestine of a CF-1 mouse (14). Strain M-2 was obtained from I. A. Holder (Shriner's Burns Institute).

Stocks of bacterial cultures were kept at −70°C in Luria broth (1% NaCl, 1% tryptone, 0.5% yeast extract [pH 7.0]) and 25% (vol/vol) glycerol. Bacterial stocks, maintained as dilute suspensions in Luria broth at 4°C, were used to inoculate cultures daily.

The standard growth medium for chemotaxis assays and growth experiments was a mineral salts mixture containing the following: dibasic potassium phosphate (0.04 M), monobasic potassium phosphate (0.02 M), ammonium sulfate (7.6×10^{-3} M), magnesium sulfate (2.0×10^{-4} M), and ferric chloride (9.2×10^{-6} M). Sodium succinate (0.4%) served as the carbon source, except where otherwise noted.

For bacterial plate counts, cells were diluted in tryptone broth and plated on tryptone plates containing 1.6% agar. Motility medium (synonomous with soft agar) contained 10 g of tryptone (Difco Laboratories, Detroit, Mich.), 3 g of yeast extract, 5 g of sodium chloride, 3 g of agar, and 1 liter of distilled water (pH 7.2).

* Corresponding author.

TABLE 1. Properties of *P. aeruginosa* strains from patients with CF

Strain	Clinical condition[a]	Colonial morphology[b]	Flagellum[c]	Motility in soft agar as determined by diam (mm) at the following times[d]:	
				6 h	24 h
M-2[e]		C	+	8	37
$437e_2$	G	R	+	7	23
572b	G	R	+	12	39
86f	G	R	+	13	50
412oo	G	R	+	9	12
320f	G	R	+	9	30
435c	G	C	+	8	35
903a	G	C	+	5	22
35c	G	C	+	5	28
902c	G	C	+	8	31
320e	G	C	+	ND	ND
412a	G	M	+	9	31
402c	P	R	+	4	20
409g	P	R	+	3	21
415gg	P	R	+	3	8
414g	P	C	+	ND	ND
503g	P	C	+	ND	ND
541a	P	C	+	5	21
144b	P	C	+	3	20
776e	P	C	+	8	23
676e	P	C	+	7	15
423mm	G	R	−	2	2
412ee	G	C	−	2	2
423gg	G	C	−	2	2
155c	P	R	−	2	2
96e	P	R	−	2	2
66g	P	R	−	2	2
414ii	P	R	−	2	2
414nn	P	R	−	2	2
503cc	P	R	−	2	2
448hh	P	R	−	2	2
448bb	P	C	−	2	2

[a] The clinical evaluation of patients is based on a modification of the scoring system of Schwachman et al. (31), in which a maximal score of 25 points is awarded for each of four categories: chest roentgenogram, general activity, physical activity, and nutrition. Patients in consultation with their physicians are then classified into three categories: 1, good (G), total score, 75 to 100; 2, moderate (M), total score 60 to 74 (no strains included); 3, poor (P), total score, less than 60.

[b] C, classic; R, rough; M, mucoid.

[c] Visible by electron microscopy; magnification, ×10,000.

[d] Two millimeters represents nonspreading colonies. ND, No data.

[e] An invasive burn strain.

Motility assay. Cells were grown for 24 h on tryptone broth agar. Several colonies were stab inoculated into the center of a plate of motility medium. For each assay, triplicate plates per strain were incubated at 37°C and examined periodically over a 1- to 24-h period for colony spreading (1). Colony diameter was measured at various time points. The average of three assays was used to graphically plot the data.

Electron microscopy. Bacteria were grown for 14 to 16 h in 5 ml of Luria broth at 37°C without shaking. Cells were stained with 0.5% phosphotungstic acid (pH 7.0). Specimens were examined with an H-600 Hitachi transmission electron microscope (magnification, ×10,000).

Capillary assay for quantitation of bacterial chemotaxis and motility. All CF strains of *P. aeruginosa* exhibiting motility in soft agar were tested by a modified Adler capillary assay (2, 24). Strain M-2 was also tested routinely as a non-CF strain to be used for comparison. The results for the CF strains could be compared relative to those for strain M-2. Strain M-2 was selected as a standard chemotactic strain. Its motility and chemotaxis are on the order of other virulent, wild-type strains such as PAO1, 1210, and 1244.

Growth experiments. A bacterial stock suspension in Luria broth (0.2 ml) was inoculated into 50 ml of mineral salts plus 0.4% succinate or 0.4% glucose and grown overnight at 37°C with rotary shaking at 175 rpm. The overnight growth was diluted with fresh medium to give an optical density (A_{590}) of 0.05 to 0.07 on a Bausch & Lomb Spectronic 20. Incubation continued at 37°C with shaking, and optical density readings (A_{590}) were taken periodically for 8 h or until an optical density at 590 nm of 1.0 was reached.

RESULTS

All CF strains were tested for motility by comparing their colony spreading with that of strain M-2 in motility agar. Nonmotile rough P strains 155c, 96e, and 66g were identified by formation of a nondiffuse colony at the site of inoculation which measured only 2 mm at 24 h (Table 1). Strains 572b and 86f (G strains; Fig. 1) displayed rapid colony spreading over a 1- to 24-h time period. P strains 402c and $437e_2$ (an auxotrophic G strain) exhibited motility that was less than one-half that of strain 86f. A plot of the spreading rate was nearly linear by up to 6 h for all strains tested (Fig. 1). Rough P strains 414ii, 414mm, 503cc, and 448hh displayed no motility in soft agar (Table 1). G strains 320f and 412oo were similar to M-2 in motility, while 415gg (P strain) was only slightly motile.

Migration of classic *P. aeruginosa* strains in motility agar was used for comparison with rough strains (Table 1). Certain classic strains were not tested in motility agar, since they were identified as nonflagellated by electron microscopy. Two G strains, 412a and 35c, displayed motility at 24 h that was similar to that of strain M-2. However, during the first 6 h, 35c motility was slightly greater than half that of M-2. G strain 902c displayed colony spreading similar to that of M-2 during the first 6 h of the assay. In contrast, both 144b and 541a (P strains) showed reduced motility compared with that of M-2. During the first 6 h of the assay, strain 144b exhibited less than half the motility of M-2, while 541a gave approximately a one-third reduction compared with that of M-2.

A single polar flagellum is one of the major taxonomic, phenotypic characteristics of *P. aeruginosa*, and its absence in clinical strains is rarely observed (20). Yet a large number of strains tested initially displayed no spreading in motility agar. The question arose as to whether these nonmotile organisms possessed flagella. To test this idea all questionable strains were examined by electron microscopy. Electron micrograph data show that 47% of the P strains lacked flagella, in contrast to the absence of flagella in only 21% of the G strains (Table 1). The absence of flagella, as demonstrated by electron microscopy, corresponded 100% with motility assay results. The absence of flagella was the most striking in those strains with rough colonial morphology. Seventy percent of the rough P strains lacked flagella, while only one rough G strain (423mm) was nonflagellated. In contrast, 80% of all classic strains examined were flagellated.

The results of more precise determinations by capillary assay of chemotactic capacity and motility are shown in Table 2. Rough strains from patients in good and poor clinical condition were examined. G strains 572b and 86f were highly chemotactic toward all three amino acids. Their

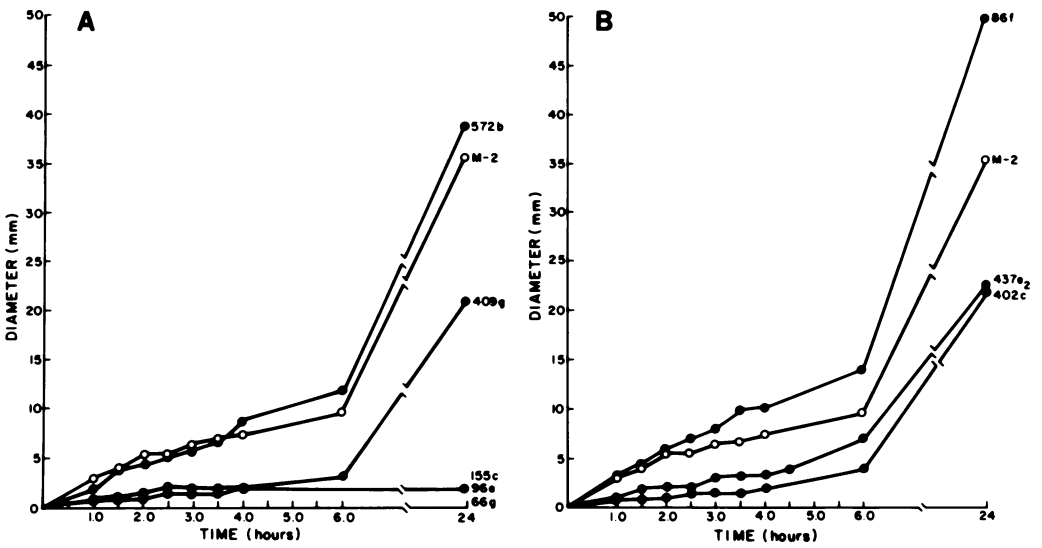

FIG. 1. (A) Migration of rough *P. aeruginosa* strains in soft agar. (B) Rough *P. aeruginosa* strains from patients with CF in good and poor clinical condition.

average response to arginine was nearly comparable to that of M-2, and their response exceeded that of M-2 for the other two attractants. Strain 412oo also exceeded M-2 in its response to serine and arginine. Of the four rough G strains tested, only 320f showed a significant reduction (69%) in response to arginine. The motility of 320f was somewhat reduced (33%) compared with that of M-2. The chemotactic response of P strain 402c (Table 2) to all three attractants was severely depressed, to an average of 13% of the value for strain M-2. The chemotactic response of strain 409g was partially lower, being 72% on average for all three attractants. This value corresponded closely to the results of the buffer (motility) capillary assay (64% that of M-2).

Three of the four classic G strains (Table 3) showed comparable responses toward arginine and alpha-aminoisobutyrate (AIB) and in their motility. Strain 902c was more chemotactic than M-2. Its response to AIB was greater than twofold that of M-2. Only the response of 435c was reduced for both attractants (10 and 34% of M-2 for AIB and arginine, respectively). This is also the only strain in this group which demonstrated significantly reduced motility (19% that of M-2) in the capillary assay. Strain 35c, although less motile (46% that of M-2), was highly chemotactic for both arginine and AIB. Apparently, motility was not the limiting factor.

Three of the four classic P strains generally were similar to M-2 in motility buffer capillary cell counts (Table 3). Only strain 541a showed reduced motility (31% that of M-2). In contrast, there was a general trend in these strains toward reduction in chemotaxis, which in some cases was severe. Strains 676e and 144b gave responses to arginine (8 and 14% that of M-2, respectively) similar to that of strain 402c, as described above. The response to AIB by strains 144b and 776e was decreased to 14% (144b) and 17% (776c) that of M-2. Data in Table 3 show that three of the four classic P strains exhibited reductions in chemotaxis to one or two amino acids.

Results of previous studies by Moulton and Montie (24) have shown that the concentration-response curve for serine showed a peak at 10^{-3} M and a peak at 10^{-2} M for arginine

TABLE 2. Motility and chemotaxis of rough CF strains of *P. aeruginosa* compared with those of M-2

Strain (clinical condition)[a]	Accumulation (10^4 CFU per capillary) in the following with the indicated concn[b]:			
	10^{-3} M Serine	10^{-2} M AIB	10^{-2} M Arginine	Buffer
572b (G)	66 ± 5	48 ± 11	53 ± 2	2.6 ± 0.7
86f (G)	49 ± 8	55 ± 9	63 ± 10	3.5 ± 0.8
412oo (G)	57	ND[c]	73 ± 5	4.1 ± 1.3
320f (G)	ND	ND	20 ± 3	1.6 ± 0.4
402c (P)	3.6 ± 1.5	2.8 ± 0.7	10 ± 2.8	1.1 ± 0.3
409g (P)	28 ± 5	28 ± 2	41 ± 5	1.7 ± 0.5
M-2	38 ± 5	29 ± 7	65 ± 2	2.4 ± 0.3

[a] Clinical condition: G, good; P, poor. For criteria, see footnote *a* to Table 1.
[b] Mean ± standard error of the mean.
[c] ND, No data.

TABLE 3. Motility and chemotaxis of classic CF strains of *P. aeruginosa* compared with those of M-2

Strain (clinical condition)[a]	Accumulation (10^4 CFU per capillary) in the following[b]:		
	AIB	Arginine	Buffer
35c (G)	35 ± 16	52 ± 8	1.1 ± 0.3
435c (G)	3.0 ± 1.1	22 ± 8	0.45 ± 0.06
902c (G)	77 ± 8	80 ± 4	3.0 ± 0.7
412a[c] (G)	20	3.6 ± 11	2.6 ± 0.25
776e (P)	4.9	24 ± 4	2.6 ± 0.25
676e (P)	ND[d]	5.2 ± 1.8	1.9 ± 0.2
144b (P)	4.1 ± 0.9	9.0 ± 4.6	1.4 ± 0.36
541a (P)	ND	82 ± 3	0.75 ± 0.32
M-2	29 ± 7	65 ± 2	2.4 ± 0.3

[a] Clinical condition: G, good; P, poor. For criteria, see footnote *a* to Table 1.
[b] Mean ± standard error of the mean.
[c] Mucoid strain.
[d] ND, No data.

TABLE 4. Taxis of *P. aeruginosa* 402c with different concentrations of amino acids[a]

Strain	Concn (M)	Accumulation (10^4 CFU per capillary) in the following:		
		AIB	Arginine	Buffer
402c	10^{-2}	0.5	0.3	ND[b]
	10^{-3}	0.6	0.6	0.3
M-2[c]	10^{-4}	0.04	0.3	ND
	10^{-2}	16.0	65.0	3.0

[a] Growth medium was mineral salts plus 0.4% succinate.
[b] ND, No data.
[c] Tested on the same day.

and AIB. Since these optimum molar concentrations of attractants were used in the capillary assays, the relative lack of 402c chemotaxis response to the amino acids could be due to the requirement of concentrations other than the ones used. Results of 402c chemotaxis toward arginine and AIB at different molar concentrations (Table 4) indicate that this was not the case. Chemotactic response of 402c was minimal for all three concentrations of arginine and AIB, while M-2, tested on the same day, showed characteristic chemotaxis and motility.

Chemotaxis of bacteria toward amino acids might be influenced by the growth medium used prior to the capillary assays. An organism unable to efficiently utilize the mineral salts medium plus succinate as a carbon source could appear to be nonchemotactic and should exhibit reduced motility. Consequently, it was important to establish whether CF strains were able to efficiently utilize the growth media required for these experiments. Experiments showed that both 86f and 66g (a nonflagellated strain) grew as well as M-2 in mineral salts medium plus succinate as a carbon source (Fig. 2). Reduced motility of 402c was not due to an inability to grow in mineral salts medium plus succinate as a carbon source (Fig. 2). The generation time of 402c was 93% that of M-2. In general, no marked differences in growth were observed for the strains tested by the chemotaxis capillary assay. We noted that growth rate is generally not correlated with chemotactic capacity (unpublished data).

A survey of the growth of all strains in mineral salts plus glucose, or succinate, compared with Luria broth showed a few metabolic deficiencies. Only three auxotrophs (23% of the total) were found in patients with all clinical conditions. Although there was some selection for auxotrophy in the lungs of patients with CF, such deficiencies could not contribute to the altered motility observed in such a large number of CF strains.

DISCUSSION

The majority of CF strains of *P. aeruginosa* examined in this study differ markedly from strains associated with acute infections with regard to motility and chemotaxis. These phenotypic parameters were stable ones not subject to alteration by laboratory environmental conditions. We hypothesize that, after initial invasion of the lungs by the organism, there may be a progressive selection against bacterial properties normally associated with invasiveness. These include motility, protease production (22), and intact lipopolysaccharide. Recent evidence is reported substantiating the lack of invasiveness of such strains in a burned mouse model (22).

The motility of the majority of CF G strains was similar to that of M-2 in soft agar. However, since a surprisingly large number of CF rough strains were nonmotile in the motility assay, we suspect that these organisms lacked flagella. Such a severe physiological aberration would be unusual in *P. aeruginosa*. The single, polar flagellum is a major taxonomic characteristic of the organism and is associated with more than 95% of clinical strains (3, 20, 25). In fact, when type-specific, thermolabile (flagellar) antigens were studied by Lanyi (20), he reported that only 1 of 541 strains was nonmotile. Detection of flagella by electron microscopy confirmed the absence of flagella in 70% of rough P strains. This severe alteration of phenotype was not limited to rough colonial morphology, since two classic strains from patients in poor clinical condition lacked flagella. However, in contrast to rough strains, 80% of classic strains from both clinical conditions were flagellated.

Deficiency in flagellation has been reported previously in a *Bacillus subtilis* mutant by Sasajima and Kumada (28, 29) and Sasajima et al. (30). A defect in the cell surface structure of the *B. subtilis* mutant caused altered cell morphology, bacteriophage sensitivity, and nonflagellation. According to

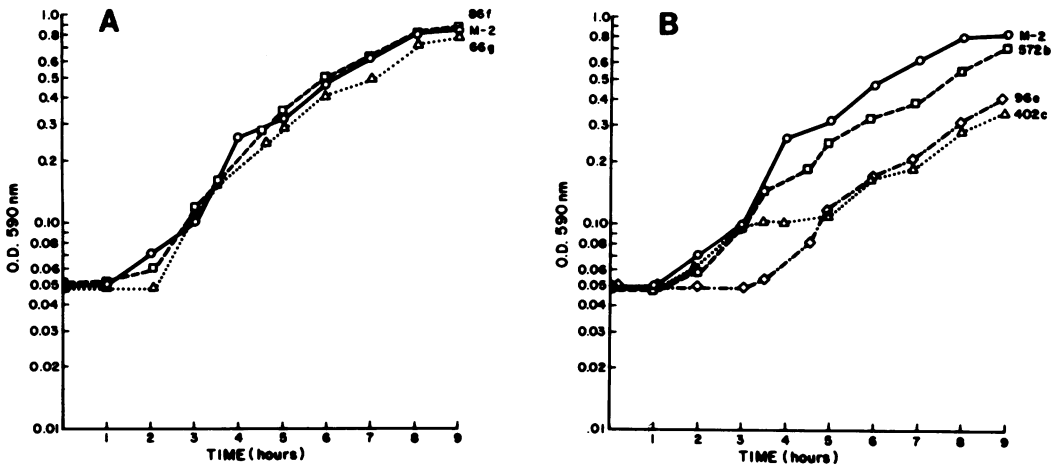

FIG. 2. (A) Growth curves of strains 86f, 66g, and M-2 grown in mineral salts medium plus succinate as a carbon source. (B) Growth curves of *P. aeruginosa* strains 572b, 96e, 402c, and M-2 grown in mineral salts medium plus succinate as a carbon source. OD_{590}, Optical density at 590 nm.

these investigators, a transketolase mutation may affect the synthesis of polysaccharide which, in turn, could alter the cell surface, resulting in an inadequate function of membrane proteins. Gram-negative mutants also have been reported which are flagellated and defective in synthesizing lipopolysaccharide (17, 18). Integration of protein in the outer membrane also has been found to be defective in a lipopolysaccharide mutant of *Escherichia coli* (17, 18). Perhaps a similar mutation event is selected for in the lungs of patients with CF, thereby favoring nonflagellated *P. aeruginosa* strains in patients who have been harboring the pathogen for prolonged periods of time. This concept seems plausible, since once established in the lungs, the organism would no longer need invasive properties such as motility and chemotaxis (22). It might be speculated further that altered synthesis of lipopolysaccharide could create envelope instability which could interfere with the secure anchoring of the flagellum. This line of thinking is supported by the fact that a basal body ring of the flagellum is associated with the lipopolysaccharide-outer membrane layer of the cells (17).

Some authors (5) have attempted to correlate mucoidy with nonflagellation by strain selection in the laboratory; however, we found no such correlation from the results of our study. Unlike the transient, mucoid phenotype, nonflagellation is not dictated by environment. Nonflagellation of *P. aeruginosa* is apparently a stable characteristic associated with specific strains. It was not altered by any variation in growth medium, temperature, or aeration. Our findings are supported by those of both Pugashetti et al. (26) and Penketh et al. (25), who have found that mucoidy is not related to other physiological characteristics such as flagellation, intact O antigen, and serum sensitivity.

A related observation concerned the chemotaxis of CF strains. There was a trend toward an association between patient clinical condition and chemotactic response, as was seen with rough strains and flagellation. Thus, six of eight strains from patients in good clinical condition showed chemotaxis comparable to or better than non-CF strain M-2, as judged by their response to one or more amino acids. In contrast four of six strains from patients in poor clinical condition were deficient (three strains showed severely reduced chemotaxis). These data, although from a much more sensitive and specific assay for chemotaxis, were in general agreement with the results from the motility plate assays.

Since rough strains from patients in poor clinical condition were predominantly nonflagellated, only two could be tested for amino acid chemotaxis by the capillary assay. Both strains were less chemotactic than strain M-2. There was a parallel reduction of motility suggesting that it is directly affected, resulting in reduced chemotaxis. As an alternative explanation, since the genetic loci for motility and chemotaxis are in close proximity (36, 37), a mutational event in that region of the chromosome could alter both gene products.

Over half of the classic strains displayed reduced chemotaxis toward one or more attractants. As in the case with rough strains, classic strains from patients in poor clinical condition were less chemotactic than those from patients in good clinical condition. While the absence of flagella was less frequently observed in classic strains, the chemotaxis results suggest that the general chemotactic response is not functioning the same as that observed in motile, chemotactic strains from acute infections. Since classic strains also show a trend toward altered lipopolysaccharide, in that 50% exhibited polyagglutination in O antigen antisera (22), this altered cell envelope could result in malfunctions such as perturbation of chemoreceptors in the membrane or of the appropriate signal transmission to the flagellum.

An interesting question is what selective pressures might be present in the lungs of patients with CF. Since the lungs of patients with CF are unique in that large amounts of mucus are secreted, especially at later stages of the disease, one would suspect that motility would be of less advantage in such a highly viscous environment. Elevation of divalent cations such as Ca^{2+} (39) could conceivably alter bacterial lipopolysaccharide synthesis as well. Since magnesium is an important requirement for integrity of the outer membrane of gram-negative bacteria (11), increased cation levels would result in outer membrane-stabilizing conditions and would select for envelope-deficient phenotypes. One approach would be to look for these changes by following in sequence respiratory tract cultures from single patients.

As mentioned above, alterations in flagella may reflect a more general problem in the cell envelope. An understanding of the nature of these changes would increase the choice and efficacy of any envelope-associated immunotherapy (14) or antibiotic chemotherapy.

ACKNOWLEDGMENT

We are indebted to Janice Allison for invaluable and expert laboratory assistance.

LITERATURE CITED

1. **Adler, J.** 1966. Chemotaxis in bacteria. Science **153**:708–716.
2. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. **74**:77–91.
3. **Ansorg, R.** 1978. Flagella specific H antigenic schema of *Pseudomonas aeruginosa*. Zentrabl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **242**:228–238.
4. **Boyce, J. R., and R. V. Miller.** 1982. Selection of nonmucoid derivatives of mucoid *Pseudomonas aeruginosa* is strongly influenced by the level of iron in the culture medium. Infect. Immun. **37**:695–701.
5. **Boyce, J. R., and R. V. Miller.** 1982. Motility as a selective force in the reversion of cystic fibrosis-associated mucoid *Pseudomonas aeruginosa* to the nonmucoid phenotype in culture. Infect. Immun. **37**:840–844.
6. **Craven, R. C., and T. C. Montie.** 1981. Motility and chemotaxis of three strains of *Pseudomonas aeruginosa* used for virulence studies. Can. J. Microbiol. **25**:458–460.
7. **Di Sant' Agnese, P. A., and P. B. Davis.** 1976. Research in cystic fibrosis. N. Engl. J. Med. **295**:597–602.
8. **Govan, J. R. W.** 1975. Mucoid strains of *Pseudomonas aeruginosa*: the influence of culture medium on the stability of mucus production. J. Med. Microbiol. **8**:513–522.
9. **Govan, J. R. W., and J. A. M. Fyfe.** 1978. Mucoid *Pseudomonas aeruginosa* and cystic fibrosis: resistance of the mucoid form to carbenicillin, flucloxacillin, and tobramycin and the isolation of mucoid variants in vitro. J. Antimicrob. Chemother. **4**:233–240.
10. **Govan, J. R. W., J. A. M. Fyfe, and C. McMillan.** 1979. The instability of mucoid *Pseudomonas aeruginosa*: fluctuation test and improved stability of the mucoid form in shaken culture. J. Gen. Microbiol. **110**:229–232.
11. **Hancock, R. E. W., L. M. Mutharia, L. Chan, R. P. Darveau, D. P. Speert, and G. B. Pier.** 1983. *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypable strains deficient in lipopolysaccharide O side chains. Infect. Immun. **42**:170–177.
12. **Hoiby, N.** 1974. *Pseudomonas aeruginosa* infection in cystic fibrosis, relationship between mucoid strains of *Pseudomonas aeruginosa* and the humoral immune response. Acta Pathol. Microbiol. Scand. Sect. B **82**:551–558.
13. **Hoiby, N.** 1977. *Pseudomonas aeruginosa* infection in cystic fibrosis. Acta Pathol. Microbiol. Scand. **262**:1–96.

14. **Holder, I. A., R. Wheeler, and T. C. Montie.** 1982. Flagellar preparations from *Pseudomonas aeruginosa*: animal protection studies. Infect. Immun. **35**:276–280.
15. **Kawaharajo, K., J. Y. Homma, Y. Aoyama, and K. Morihara.** 1975. In vivo studies on protease and elastase from *Pseudomonas aeruginosa*. Jpn. J. Exp. Med. **45**:89–100.
16. **Klinger, J. D., D. C. Straus, C. B. Hilton, and J. A. Bass.** 1978. Antibodies to proteases and exotoxin A of *P. aeruginosa* in patients with cystic fibrosis: demonstration of radioimmunoassay. J. Infect. Dis. **138**:49–58.
17. **Komeda, Y., T. Icho, and T. Iino.** 1977. Effects of *galU* mutation on flagellar formation in *Escherichia coli*. J. Bacteriol. **129**:908–915.
18. **Koplow, J., and H. Goldfine.** 1974. Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. **117**:527–543.
19. **Lam, J., R. Chan, K. Lam, and J. W. Costerton.** 1980. Production of mucoid microcolonies by *Pseudomonas aeruginosa* within the infected lungs in cystic fibrosis. Infect. Immun. **28**:546–556.
20. **Lanyi, B.** 1970. Serological properties of *Pseudomonas aeruginosa*. II. Type-specific thermolabile (flagellar) antigens. Acta Microbiol. Acad. Sci. Hung. **17**:35–48.
21. **Laraya-Cuasay, L. R., K. R. Cundy, and N. N. Huang.** 1976. *Pseudomonas* carrier rates of patients with cystic fibrosis and of members of their families. J. Pediatr. **89**:23–26.
22. **Luzar, M. A., and T. C. Montie.** 1985. Avirulence and altered physiological properties of cystic fibrosis strains of *Pseudomonas aeruginosa*. Infect. Immun. **50**:572–576.
23. **May, J. R., N. C. Herrick, and D. Thompson.** 1972. Bacterial infection in cystic fibrosis. Arch. Dis. Child. **47**:908–913.
24. **Moulton, R. C., and T. C. Montie.** 1979. Chemotaxis by *Pseudomonas aeruginosa*. J. Bacteriol. **137**:274–280.
25. **Penketh, A., T. Pitt, D. Roberts, M. E. Hodson, and J. C. Batten.** 1983. The relationship of phenotype changes in *Pseudomonas aeruginosa* to the clinical condition of patients with cystic fibrosis. Am. Rev. Respir. Dis. **127**:605–608.
26. **Pugashetti, B. K., H. M. Metzger, J. J. Cardamone, and D. E. Feingold.** 1983. Flagellation is a strain-dependent characteristic of cystic fibrosis-associated mucoid strains of *Pseudomonas aeruginosa*. Eur. J. Clin. Microbiol. **2**:360–361.
27. **Reynolds, H. Y., P. A. Di Sant' Agnese, and C. H. Zierdt.** 1976. Mucoid *Pseudomonas aeruginosa*. J. Am. Med. Assoc. **232**:2190–2192.
28. **Sasajima, K., and T. Kumada.** 1981. Change in the regulation of enzyme synthesis under catabolite repression in *Bacillus subtilis* pleiotropic mutant lacking transketolase. Agric. Biol. Chem. **45**:2005–2012.
29. **Sasajima, K., and T. Kumada.** 1983. Deficiency of flagellation in *Bacillus subtilis* pleiotropic mutant lacking transketolase. Agric. Biol. Chem. **47**:1375–1376.
30. **Sasajima, K., T. Kumada, and A. Yokota.** 1977. A pleiotropy in carbohydrate metabolism of *Bacillus subtilis* mutant lacking transketolase. IFO Res. Commun. **8**:69–77.
31. **Schwachman, H., L. L. Kulczycki, and K. T. Khaw.** 1965. Studies in cystic fibrosis: a report on sixty-five patients over 17 years of age. Pediatrics **36**:689–699.
32. **Seale, T. W., H. Thirkill, M. Tarpay, M. Flux, and O. M. Rennert.** 1979. Serotypes and antibiotic susceptibility of *Pseudomonas aeruginosa* isolates from single sputa of cystic fibrosis patients. J. Clin. Microbiol. **9**:72–78.
33. **Snell, K., I. A. Holder, S. A. Leppla, and C. B. Saelinger.** 1978. Role of exotoxin and protease as possible virulence factors in experimental infections with *Pseudomonas aeruginosa*. Infect. Immun. **19**:839–845.
34. **Stratford, B. C., and S. Dixson.** 1974. Serum levels of gentamicin and tobramycin after slow intravenous bolus injection. Lancet **ii**:378–379.
35. **Thomassen, M. J., and C. A. Demko.** 1981. Serum bactericidal effect on *Pseudomonas aeruginosa* isolates from cystic fibrosis. Infect. Immun. **33**:512–518.
36. **Tsuda, M., and T. Iino.** 1983. Ordering of the flagellar genes in *Pseudomonas aeruginosa* by insertions of mercury transposon T 501. J. Bacteriol. **153**:1008–1017.
37. **Tsuda, M., and T. Iino.** 1983. Transductional analysis of flagellar genes *Pseudomonas aeruginosa*. J. Bacteriol. **153**:1018–1026.
38. **Wahba, A. H., and J. H. Darrell.** 1965. The identification of atypical strains of *Pseudomonas aeruginosa*. J. Gen. Microbiol. **38**:329–342.
39. **Wood, R. E., T. F. Boot, and C. F. Doershuk.** 1976. Cystic fibrosis. Am. Rev. Respir. Dis. **113**:833–878.